Studies on the Metabolism of Semen

6. ROLE OF HORMONES. EFFECT OF CASTRATION, HYPOPHYSECTOMY AND DIABETES. RELATION BETWEEN BLOOD GLUCOSE AND SEMINAL FRUCTOSE

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The function of the male accessory organs of reproduction is to elaborate the seminal plasma. This activity is subject to a strict control by various hormones, particularly those produced in the testes and the anterior pituitary. Knowledge concerning the relationship between hormones and the functional state of the male accessory organs was provided mainly by anatomical and histological studies. The so-called 'hormone indicator tests' were elaborated from the information gained by these researches. Recently a successful attempt was made to use as indicator tests two chemical reactions, the 'fructose test' and 'citric acid test', based upon the finding that the ability of the accessory glands to produce fructose and citric acid depends upon the activity of the testicular hormone. Both fructose and citric acid were shown to disappear from the seminal plasma as the result of castration and to reappear promptly after implantation or injection of testosterone (Mann & Parsons, 1947; Mann, Lutwak-Mann & Price, 1948; Mann, Davies & Humphrey, 1949).

This paper contains results concerning the quantitative aspects of the relation between testosterone and the secretion of fructose and citric acid in the male accessory organs of reproduction. New findings will be reported, obtained in experiments with hypophysectomized animals, which show that the endocrine influence of the testes on the chemical constituents of semen is integrated with the functioning of the anterior pituitary gland. Another object of the present study was to investigate the part played by yet another hormone, insulin, in the endocrine control of semen composition; experiments will be described, carried out with alloxan-diabetic rabbits, which point to the existence of a definite relationship between the level of fructose in semen and that of glucose in blood (preliminary communication, Mann & Parsons, 1949).

METHODS

In experiments on rabbits, the experimental material consisted of semen and male accessory organs. The collections of semen were carried out at definite time intervals by means of an artificial vagina fitted out with a glass cup receptacle of known weight (Macirone & Walton, 1938). Immediately after ejaculation the glass cup containing the semen was detached from the rubber portion of the vagina and weighed; the amount of the two major components of rabbit semen, gel and fluid, was then quickly measured. Finally the semen was deproteinized by grinding with trichloroacetic acid (final concentration 8% w/v). The accessory organs, obtained by dissection, consisted of glandula seminalis, glandula vesicularis and prostate. Both young and fully mature normal bucks were used. The castration was performed usually so as to remove only the testes, leaving the epididymes intact. Hypophysectomies were carried out in rabbits by Dr G. W. Harris using the technique of Jacobsohn & Westman (1940). Diabetes was produced in rabbits by injecting alloxan intravenously. Only such rabbits were retained for experimental purposes in which the level of seminal fructose before the alloxan treatment was fairly constant, and in which alloxan did not produce toxic symptoms such as loss of weight, fall in sexual drive or decrease in the volume of semen ejaculates. Best results were obtained by giving a rabbit an injection of 75 mg. of alloxan/kg. body weight, and repeating this dose 1 or 2 days later.

In experiments on rats the following organs were used for analysis: seminal vesicle, coagulating gland, ventral prostate and dorsolateral prostate. The rats were killed under ether anaesthesia in order to prevent ejaculation and loss of secretory fluid from the accessory organs of reproduction. Unless otherwise stated in the text, the organs were analysed together with the secretions contained in them. Immediately after dissection the organs were ground with trichloroacetic acid (final concentration 8% w/v) and protein-free extracts obtained by centrifugation. Castration was performed in rats through the scrotum, and the epididymes removed together with both testes.

Fructose was determined colorimetrically; glucose was estimated by means of glucose oxidase, as the difference in the total reducing sugar content before and after aerobic incubation with the enzyme (Mann, 1946, 1948). Citric acid was determined by the method of Speck, Moulder & Evans (1946).

RESULTS

Content of fructose, glucose and citric acid in normal rabbit semen

A single ejaculate of rabbit may vary in volume from less than 1 ml. to as much as 6 ml., but when the two main portions of the semen, gel and fluid, are

measured separately it will be found that it is mainly the quantity of gel which is subject to very considerable variation whereas the fluid portion is more constant. This circumstance has an important bearing on the measurements of fructose and citric acid in rabbit semen. It explains why fructose, which is chiefly associated with the fluid portion, does not vary in quantity from one ejaculate to another nearly as much as citric acid which is found largely in the gel portion. Fig. 1, which represents the record of weekly collections of semen from a single rabbit over a period of 16 weeks, is given as an illustration of individual variations in seminal fructose were established between different rabbits. Parsons (1950) has recently analysed seventy-two ejaculates collected from twelve different normal rabbits; the average values (each representing an average from six ejaculates collected at weekly intervals) covered a range from 280 to 962 μg./ejaculate or 40–420 mg./100 ml. of semen.

The present study has brought to light a new fact, namely, that rabbit semen, unlike that of bull, ram and man, occasionally contained an appreciable, but variable, admixture of glucose in addition to fructose. The glucose content was sometimes low or negligible but some ejaculates contained as much as 400 μg. This fact will be described more fully in connexion with the action of insulin on the composition of semen in diabetic animals (Table 5).

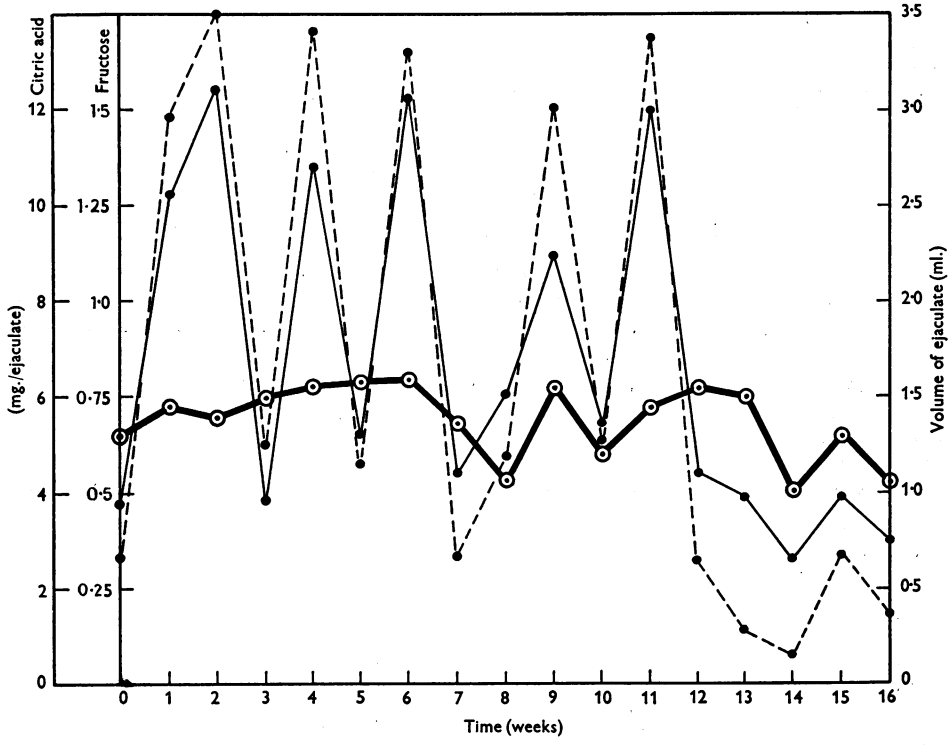

Fig. 1. Relation between volume (●—●—●) of ejaculate and content of fructose (⊙—⊙—⊙) and citric acid ●- -●- -● in rabbit semen.

this phenomenon. The animal from which this record was obtained was a normal fully mature buck, but with exceptionally large fluctuations in the volume of ejaculates. The volume fluctuated in the same direction as the content of citric acid, with fructose remaining relatively stable. The difference in the behaviour of fructose and citric acid becomes obvious, however, only if the analytical results are considered in terms of absolute quantities (mg. or μg./ejaculate) and not merely as concentrations (mg./100 ml.). However, the content of fructose was by no means equally stable in all the rabbits investigated; it was not unusual to find rabbits with fairly wide day-to-day fluctuations. Considerable

Effect of castration and testosterone

The effects of castration and testosterone were studied on rabbits and rats. In rabbits the semen and accessory organs become largely depleted of fructose within 2–3 weeks after castration (Mann & Parsons, 1947); there is also a sharp decline in the amount of gel and citric acid (Humphrey & Mann, 1949). Results presented in Table 1 are the outcome

Table 1. *Effect of castration and testosterone on the semen and accessory organs of reproduction in the rabbit*

	Semen					
			Fructose		Citric acid	
	Average from animals (no.)	Volume (ml.)	(mg./ 100 ml.)	(μg./ ejaculate)	(mg./ 100 ml.)	(μg./ ejaculate)
Normal buck	10	1·3	55	720	210	2750
2 weeks after castration	4	0·4	35	140	80	320
3 weeks after castration	4	0·3	3	9	15	45
6 weeks after castration and simultaneous implantation of testosterone (100 mg.)	4	2·0	34	680	150	3000
6 weeks after castration and simultaneous implantation of testosterone, pellet removed and rabbit examined 3 weeks later	2	0·2	5	10	30	60

	Accessory organs with their secretory fluids										
		Glandulae vesicularis and seminalis					Prostate				
			Fructose		Citric acid			Fructose		Citric acid	
	Average from animals (no.)	Weight (mg.)	(mg./ 100 g.)	(μg./ organ)	(mg./ 100 g.)	(μg./ organ)	Weight (mg.)	(mg./ 100 g.)	(μg./ organ)	(mg./ 100 g.)	(μg./ organ)
Normal buck	4	930	45	420	328	3050	870	120	1040	50	440
2 weeks after castration	2	610	5	32	70	430	520	19	100	3	34
3 weeks after castration	3	480	2	10	18	86	420	3	13	1	4
6 weeks after castration and simultaneous implantation of testosterone (100 mg.)	2	1200	29	350	210	2540	1160	87	1020	38	440
6 weeks after castration and simultaneous implantation of testosterone, pellet removed and rabbit examined 3 weeks later	2	800	4	32	25	200	930	14	130	2	18

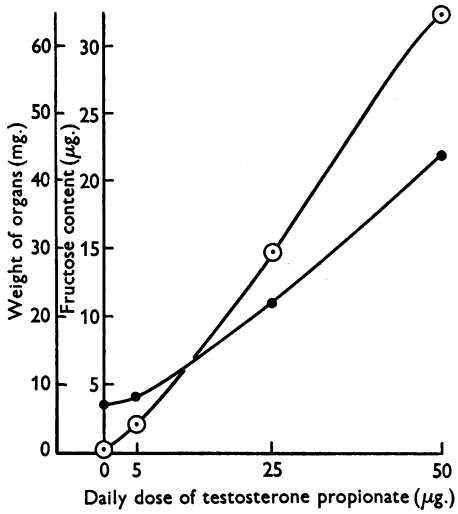

Fig. 2. Dosage-response curves of testosterone propionate, using the coagulating glands of the rat; ⊙—⊙—⊙, fructose (μg.); ●—●—●, weight of organs (mg.).

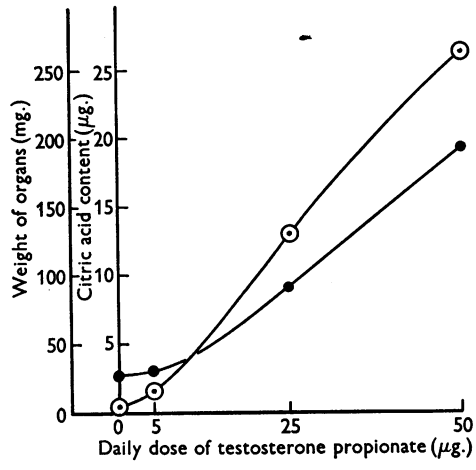

Fig. 3. Dosage-response curves of testosterone propionate, using the seminal vesicles of the rat; ⊙—⊙—⊙, citric acid (μg.); ●—●—●, weight of organs (mg.).

Table 2. *Effect of castration and testosterone on male accessory organs of the rat*

Condition of rats	Average from animals (no.)	Coagulating glands			Dorsolateral prostate			Seminal vesicles			Ventral prostate		
			Fructose			Fructose			Citric acid			Citric acid	
		Weight (mg.)	(μg.)	(mg./100 g.)	Weight (mg.)	(μg.)	(mg./100 g.)	Weight (mg.)	(μg.)	(mg./100 g.)	Weight (mg.)	(μg.)	(mg./100 g.)
Normal:													
1-year old, average weight 430 g.	3	230	293	127	440	170	38	1590	970	57	750	293	39
Castrated when 3 weeks old:													
Autopsied when 1 year old	6	3	0	0	6	0	0	6	0	0	8	0	0
Left for 11 months, then implanted with 100 mg. of testosterone; autopsied 5 weeks later	2	193	402	207	523	345	66	1413	1150	80	334	350	105
Left for 1 year, then injected with testosterone propionate as follows:													
8 injections of 50 μg. daily	2	15	18	120	40	20	50	159	120	75	50	30	60
30 injections of 50 μg. daily	2	40	90	225	90	85	94	320	390	122	178	115	65
Castrated when 11 months old:													
Autospied 7 weeks later	3	30	1	3	75	3	4	135	25	18	83	23	27
Left 4 weeks, then injected with testosterone propionate:													
21 injections of 200 μg. daily	2	260	293	113	470	280	60	1250	680	54	700	320	46

Table 3. *Effect of testosterone propionate on normal rats*

Rats (4 months old)	Weight of rat (g.)	Weight of organs (mg.)						Fructose (μg.)		Citric acid (μg.)	
		Testis (one)	Epididymis (one)	Coagulating glands	Dorsolateral prostate	Seminal vesicles	Ventral prostate	Coagulating glands	Dorsolateral prostate	Seminal vesicles	Ventral prostate
Non-treated	215	932	327	84	129	187	152	64	70	50	29
	215	1026	335	48	152	180	195	64	64	27	63
	230	938	313	37	134	192	245	58	69	37	175
30 injections of 200 μg. daily, prior to autopsy	205	748	354	200	413	1058	474	355	251	305	381
	240	859	436	166	376	1376	585	255	300	870	910

of a combined study of both semen and accessory organs in a group of buck rabbits of similar age (16–22 months) and weight (2·7–3·2 kg.); they show the close dependence between the male sex hormone and the secretory function of the reproductive organs, as expressed in the fructose and citric acid content. Testosterone implanted immediately after castration prevented the post-castration fall of fructose as well as of citric acid, but when the implant was removed there was a prompt fall in the level of both substances. On the other hand, the post-castration reduction in the volume of the ejaculates, and in the weight of the reproductive organs, were not nearly as large or rapid as the change in the output of fructose and citric acid.

In rats the response to castration and testosterone is essentially the same as in rabbits. Much depends, however, on the age of the rats at castration, the period allowed for post-castration regression, the length of time during which the hormone treatment is continued and the method of application (implantation or injection); allowance should also be made for considerable individual variations in the response to testosterone even in rats of the same age and strain.

The following experiment was set up to study the relationship between the daily injected dose of testosterone propionate and the level of fructose and citric acid in the rat accessory organs. Twenty-six rats were castrated when 7 weeks old, left for 8 weeks, and then 4 groups of 8, 8, 6 and 4 animals injected daily for 3 weeks with 0, 5, 25 and 50 μg. of testosterone propionate respectively. At autopsy the material dissected from rats of the same group was pooled, weighed, deproteinized, analysed, and the results calculated in μg. of fructose and citric acid per animal. Figs. 2 and 3 illustrate the results obtained with two organs, the coagulating gland and the seminal vesicle. These two organs were chosen because it was previously shown by Humphrey & Mann (1949) that in the rat, fructose is formed in the coagulating gland, citric acid in the seminal vesicle. The results for each gland are expressed by two dosage-response curves obtained by plotting the arithmetic dose of the hormone against the weight of the organ and the contents of fructose and citric acid respectively. As can be seen from both Figs. 2 and 3, the chemical secretory response to testosterone was proportionally much greater than the hormone-induced increase in weight of organs. For instance, the smallest dose of hormone, 5 μg. of testosterone propionate per day, caused in the castrated rats a rise in fructose and citric acid from 0·3 to 2·0 μg. and from 0·4 to 1·5 μg. respectively, whereas the corresponding increases in weight were from 7 to 8 mg. in the coagulating glands, and from 26 to 29 mg. in the seminal vesicles.

The dependence of the activity of the accessory glands upon the male sex hormone was further demonstrated on rats which were castrated when they were only 3 weeks old, that is before their accessory organs began to produce either fructose or citric acid. In such rats, left untreated for a year, the accessory organs rapidly responded to treatment with testosterone as shown by the amounts of fructose and citric acid secreted by them (Table 2).

Normal, 4-month-old rats, injected with massive doses of the male hormone (200 μg. of testosterone propionate daily) responded by increasing the level of fructose and citric acid formation above that of the normal non-treated controls (Table 3). However, when the injections were extended, the state of overstimulation in the accessory organs was accompanied by a marked decline in the size of the testes; after 7 weeks' treatment with 200 μg. of testosterone propionate per day the reduction in the weight of testes was nearly 50 %.

Effect of hypophysectomy and gonadotrophin

Hypophysectomy produced effects similar to castration. The hypophysectomized rabbit responded to the subcutaneous implantation of a pellet of testosterone (100 mg.) with renewed secretion of fructose and citric acid. When, instead of testosterone, the pregnant-mare serum gonadotrophin ('Gestyl', Organon) was used, the accessory organs responded in a similar manner (Table 4). It was interesting to note that the effect obtained with gonadotrophin was more pronounced with regard to citric acid (glandula vesicularis) than fructose (prostate). However, it would be necessary to confirm this observation on a larger number of experimental animals before it could be considered significant.

Relationship between glucose in blood and fructose in semen

It was found in an earlier experiment that when a normal animal was rendered severely hypoglycaemic with insulin for a short period of time (11 hr.), the content of fructose in accessory glands was not markedly changed (Mann *et al.* 1949). Clearly, to tackle the problem of the relationship between blood glucose and seminal fructose it was essential (1) to establish experimental conditions which would ensure not merely transient but more lasting increases or decreases in the level of blood sugar, and (2) to carry out a sufficiently large number of semen collections and fructose analyses in order to exclude results due to normal fluctuations. To satisfy these requirements several methods were tried, but the most satisfactory results were obtained in the following manner. The extent of normal variations of seminal fructose in a given animal was first established in a series of estimations, then the animal was rendered diabetic with alloxan, and the estimations

of blood sugar and seminal fructose continued for several more weeks or months. Fig. 4 illustrates the course of such an experiment performed on a rabbit from which regular collections of blood and semen of individual ejaculates, as can be seen, for instance, from Table 5. As a matter of fact, however, in the case of rabbit-seminal fructose both methods of presentation, concentration (mg./100 ml.) and con-

Table 4. *Influence of hypophysis on the formation of fructose and citric acid in male accessory organs*

Buck rabbit	Glandula vesicularis			Prostate		
	Weight (mg.)	Fructose (μg./ organ)	Citric acid (μg./ organ)	Weight (mg.)	Fructose (μg./ organ)	Citric acid (μg./ organ)
1. Normal	930	864	2840	810	880	430
2. 4 weeks after hypophysectomy	159	24	40	149	10	6
3. 4 weeks after hypophysectomy and simultaneous implantation of testosterone	1190	208	840	1360	930	410
4. 6 weeks after hypophysectomy; for the last 4 weeks injected with 200 i.u. Gestyl* daily	1140	165	8280	526	210	395
5. 4 weeks after castration	350	5	15	280	20	10
6. 4 weeks after castration and simultaneous implantation of testosterone	1200	730	2180	1080	1350	210

* Pregnant-mare serum gonadotrophin, Organon.

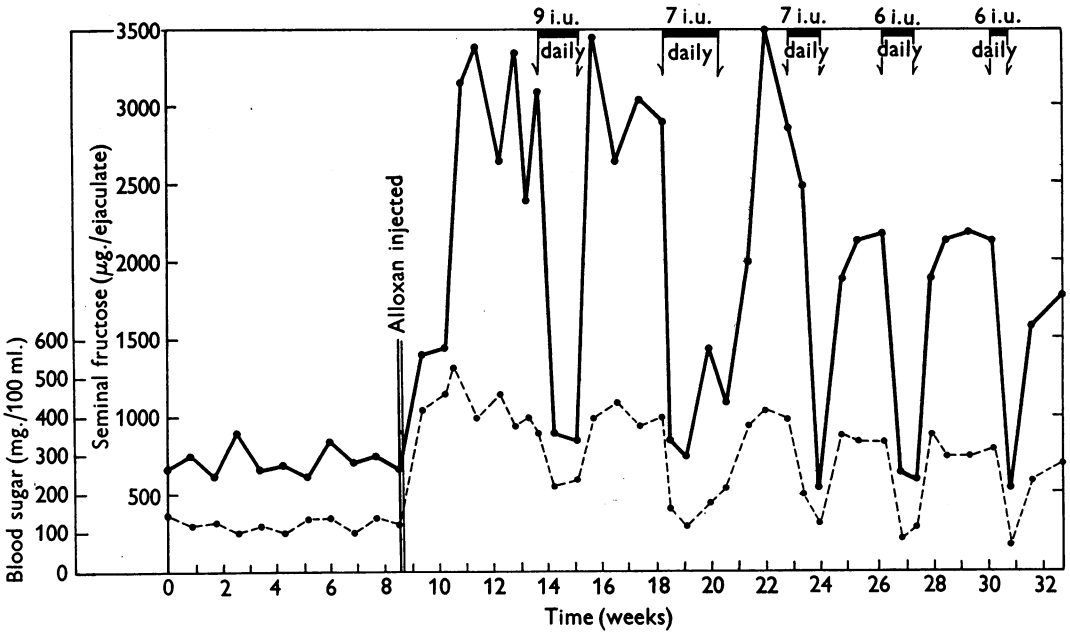

Fig. 4. Effect of alloxan diabetes and insulin on seminal fructose in the rabbit. The periods of insulin treatment and daily dose (in units, i.u.) are indicated by arrows; ●—●—●, seminal fructose, ●- -●- -●, blood sugar.

were carried out during a period of 7 months. It may be noted in Fig. 4 that whereas the blood sugar values are expressed in mg./100 ml., those relating to fructose in semen are given in μg./ejaculate and not in terms of concentration. This method was adopted because it brings out more clearly the quantitative difference in the chemical composition tent (μg./ejaculate), yield similar curves so long as the concentration of fructose in semen is given in mg./100 ml. of the fluid portion of the semen and not of the whole semen, which includes variable amounts of gel. In the buck used for the experiment illustrated in Fig. 4 the volume of the fluid portion varied not more than from 0·8 to 1·2 ml. This animal had,

before the alloxan injections, a blood sugar content of 100 mg./100 ml. and of fructose in semen about 700 μg./ejaculate or 70 mg./100 ml.; as a result of alloxan treatment this rabbit developed glucosuria and its blood sugar level rose to 400–550 mg./100 ml. At the same time there was an increase in seminal fructose which reached a peak value of 3000 μg./ejaculate or 300 mg./100 ml. at about 3 weeks after the injections of alloxan. When it was satisfactorily established that repeated collections of semen from the diabetic animal continue to yield ejaculates with an abnormally high fructose content, a study was commenced of the effect of insulin on the diabetic animal. Insulin was injected three times daily in

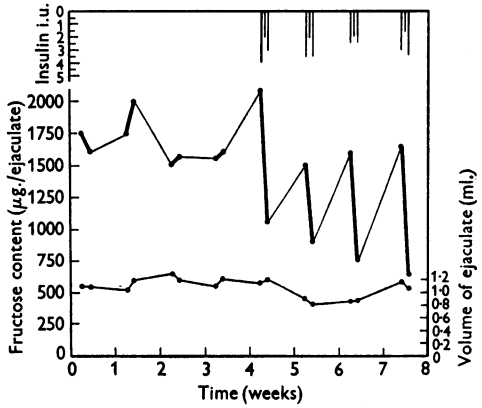

Fig. 5. Effect of insulin treatment of short duration on seminal fructose in the diabetic rabbit. Collections of semen made on the first and second day of every week: upper curve, ●—●—●, fructose; lower curve, ●—●—●, volume.

quantities sufficient to bring the blood sugar level down to 100–200 mg./100 ml. within 2 hr. after each injection. For this purpose relatively large doses of insulin were required at first, up to 10 units/day. A few weeks later, however, when the treatment was repeated, the same effects could be produced with smaller doses of insulin. As regards the behaviour of fructose, it can be seen from Fig. 4 that simultaneously with the insulin-induced fall in blood glucose, there was always a big reduction in the fructose content of semen, and that when the effect of insulin on blood glucose wore off, fructose in semen also rose.

In order to find out how soon after insulin administration the level of seminal fructose is affected, the following experiment was performed with an alloxan-diabetic rabbit (Fig. 5). For 8 weeks in succession, two collections of semen were made each week on the first and second day respectively. Without insulin the two ejaculates on the two successive days differed very little from each other with respect to volume or fructose content. However, if the rabbit was subjected to treatment with insulin immediately after the first collection, then the next day the fructose content of semen was considerably lowered although the volume of semen remained unchanged.

In another experiment two littermate buck rabbits were used; one was rendered diabetic with alloxan, the other remained as untreated control. The experiment summarized in Fig. 6 shows that alloxan diabetes has produced a very distinct increase in the output of fructose in semen. In this particular experiment, however, the degree of variation in the fructose content was much more pronounced in the

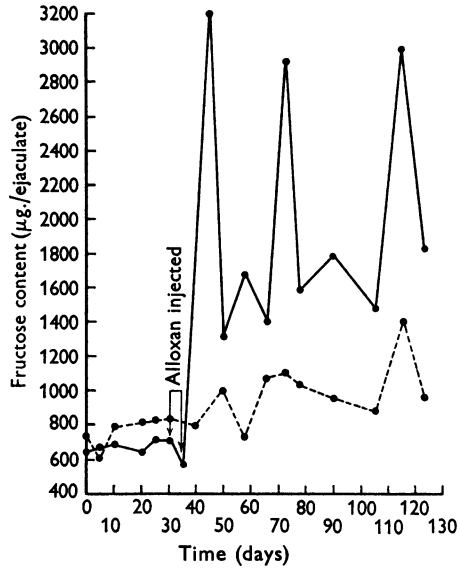

Fig. 6. Fructose content of semen in littermate rabbits, normal and alloxan-diabetic; ●—●—●, diabetic rabbit; ●- - -●- - -●, control littermate.

diabetic buck than in the control littermate, and occasionally the diabetic animal yielded ejaculates with a nearly normal sugar content.

The level of citric acid, unlike that of fructose, was not markedly affected by alloxan diabetes or by insulin. On the other hand, the level of seminal glucose was definitely increased in diabetic rabbits and it responded to insulin treatment in a manner similar to fructose. This can be seen from Table 5 which offers also a comparison between the level of glucose in blood and that of fructose in semen. It is essential to point out, however, that the results recorded in Table 5 were based on analyses of ejaculates collected at about 3-day intervals. Different results would have been obtained if the semen collection had been more frequent. Table 6 records several experiments (*A* 1, *B* 1, *C* 1, *D*, *E*, *F* and *G*) in which ejaculates were obtained both from normal and from diabetic rabbits at very short and frequent time intervals. From these it can be seen

that as a result of frequently repeated semen collections the citric acid level came down very considerably; moreover, the depletion of citric acid went further in the diabetic than in the normal control animal. It may also be noted from Table 6 that, due to great frequency in semen collections, there was a much sharper decline in the volume of semen and its fructose content in the diabetic than in the normal rabbit. These effects must be attributed to the generally lower rate of semen regeneration in diabetic individuals than in healthy normal males.

1180 mg./100 ml., and 40·6, 37·6, 35·3 and 42·6 mg./ejaculate. Harvey (1948), in her recent survey of fructose in 150 specimens of human semen, recorded values ranging from 5 to 640 mg./100 ml. or 0·05 to 31·6 mg./ejaculate. Even if due allowance is made for the very large variations in the fructose level of normal human semen, the fructose values found in the diabetic semen doubtless exceed the normal level.

Table 5. *Effect of diabetes and insulin on the content of fructose, glucose and citric acid in rabbit semen*

	Semen							
		Fructose		Glucose		Citric acid		Blood sugar
Days (no.)	Ejaculate (ml.)	(μg./ ejaculate)	(mg./ 100 ml.)	(μg./ ejaculate)	(mg./ 100 ml.)	(μg./ ejaculate)	(mg./ 100 ml.)	(mg./ 100 ml.)
1	0·65	635	97	210	32	3850	587	132
4	0·16	630	400	120	78	670	425	134
7	2·07	740	36	0	0	12100	584	116*
10	1·42	760	54	42	3	456	32	125
13	Injected intravenously alloxan (75 mg./kg.)							
14	Injected intravenously alloxan (75 mg./kg.)							
17	0·67	1700	253	310	46	3650	545	485†
20	1·24	3540	285	780	63	13200	1064	492
23	0·63	1280	205	137	22	3020	479	272
26	3·03	3280	108	1665	55	14100	465	434
29	1·29	2980	231	890	69	4040	313	340
30–33	Injected daily with 10 units of insulin							
34	3·20	790	25	10	0·3	15800	493	135
37	1·05	3450	329	650	62	12100	1152	515
40	3·80	1790	47	460	12	29100	765	425
41–43	Injected daily with 8 units of insulin							
44	1·09	850	77	0	0	7000	642	149

* Glucose (89 mg. as estimated by glucose oxidase) + 2 mg. of fructose (colorimetrically) + 26 mg. of reducing, non-fermentable material.

† Glucose (469 mg.) + 2 mg. of fructose + 14 mg. of reducing, non-fermentable material.

Several experiments were carried out to investigate the effect of intravenously introduced substances such as glucose, fructose, sucrose and Na pyruvate, upon the rate of fructose generation in the male reproductive organs. In these experiments, however, with the exception of fructose itself, none of the injected substances affected the output of fructose in semen. However, even in the case of fructose one must not overlook the possibility that the observed increase in seminal fructose may have been at least partly due to general flooding with fructose of tissues and body fluids, as shown by the excretion of a fairly large proportion of the injected fructose through the kidneys.

Although our results concerning the relationship between blood glucose and seminal fructose have been so far confined to rabbit, it appears that similar conditions also prevail in man. In four samples of semen from three diabetic patients the following values for fructose were found: 765, 940, 820 and

DISCUSSION

The composition of seminal plasma, unlike that of other body fluids, is subject to considerable fluctuations, even in the same animal. As a mixture of secretions produced by several accessory organs of reproduction, the seminal plasma varies according to the relative contribution made by the organs concerned. In this connexion the actual size and 'storage' capacity of each of these glands are, of course, of great importance. Another determining factor is the influence of the male sex hormone upon the capacity of the accessory glands to produce characteristic constituents of semen, such as fructose and citric acid (Mann & Parsons, 1947; Humphrey & Mann, 1949). Based on this observation the estimation of fructose and citric acid were elaborated into a 'hormone indicator test' of considerable sensitivity and at the same time of great simplicity. It was shown earlier by Mann *et al.* (1949) that in castrated bull calves the early effects of an implantation of testosterone can be recognized much more easily through the large increase in the content

Table 6. *Effect of intravenously injected carbohydrate on the content of fructose and citric acid in semen*

Rabbit	Exp. no.	Injected substance	Time after injection (hr.)	Semen: Ejaculate (g.)	Semen: Fructose (μg./ejaculate)	Semen: Fructose (mg./100 g.)	Semen: Citric acid (μg./ejaculate)	Semen: Citric acid (mg./100 g.)	Urine. Sugar excreted in 24 hr.	Blood. Reducing sugar (mg./100 ml.)
A (normal)	1	None	0	1·48	1080	73	7830	535	—	—
			2	0·64	1280	200	400	62	—	—
			6	0·59	450	76	250	42	—	—
			24	1·26	780	62	2150	170	—	—
	2	Glucose (10 g.)	0	1·09	980	90	5700	522	Glucose, 0·7 g.	124
			2	0·40	900	224	287	72		238
			6	0·51	1230	241	610	120		134
			24	1·19	870	73	1230	103		138
B (normal)	1	None	0	3·25	1210	37	16350	500	—	—
			2	1·00	1420	142	520	52	—	—
			6	0·30	1080	360	520	173	—	—
			24	1·66	630	38	3780	227	—	—
	2	Sucrose (12 g.)	0	2·51	1060	42	20200	800	—	136
			4	0·40	780	194	450	112	—	158
	3	Fructose (10 g.)	0	0·90	1090	121	3150	350	Fructose, 1·2 g.	130
			4	0·30	3250	1083	215	71		216
			24	0·31	760	245	430	139		125
	4	Fructose	0	2·04	1190	59	11600	570	Fructose, 1·3 g.	—
			4	0·90	8610	957	515	57		—
			24	0·76	1570	206	2450	—	—	—
C (diabetic)	1	None	0	1·44	2960	206	10500	322	Glucose, 9 g.	385
			4	0·50	1130	226	1060	212		410
			24	0·98	795	81	1150	118		360
	2	Glucose (10 g.)	0	3·04	1920	63	15400	502	Glucose, 16 g.	224
			4	1·00	1150	115	1750	175		231
			24	1·40	520	37	6500	465		385
	3	Fructose (10 g.)	0	3·30	2450	74	11200	370	—	365
			4	1·21	2700	223	1950	161	—	382
			24	1·60	1080	67	3700	231	—	333
	4	Na pyruvate* (2 g.)	0	1·41	1940	137	4640	330	—	—
			4	0·55	610	111	900	164	—	—
			24	1·39	1020	73	4750	342	—	—
D (diabetic)	1	None	0	1·71	2300	134	13100	766	—	419
			2	0·50	1285	257	450	90	—	—
			6	0·10	20	20	52	52	—	385
E (normal)	1	None	0	3·09	875	28	3310	107	—	129
			2	1·28	778	61	1640	128	—	—
			6	0·72	540	75	750	104	—	134
F (normal)	1	None	0	2·45	1210	49	11600	474	—	—
			2	0·95	1700	179	515	54	—	—
			6	1·03	780	75	1115	111	—	—
G (diabetic)	1	None	0	0·89	1880	210	8650	970	—	—
			2	0·39	620	160	890	228	—	—
			6	0·10	20	20	20	20	—	—

* The 3 ejaculates contained 0, 41 and 154 μg. of pyruvic acid, respectively.

of fructose and citric acid in the seminal glands than the rather insignificant histological recovery changes in the same glands. An example of the intimate relationship between male hormone and the secretion of fructose and citric acid was also provided by experiments in which rat accessory gland tissues completely separated from the rest of the male reproductive tract and transplanted subcutaneously into male or female hosts responded readily to testosterone by producing fructose and citric acid (Mann *et al.* 1948; Lutwak-Mann, Mann & Price, 1949). The determination of fructose and citric acid was also made use of to define the degree of androgenic activity even when this activity is relatively slight; thus, for instance, it has been applied successfully to the study of the androgenic activity of intact ovaries,

and of pure progesterone in rat (Price, Mann & Lutwak-Mann, 1949).

In the present study of the relationship between the testicular hormone and the chemical processes of secretion in the accessory organs, the quantitative aspects have been examined. The experiments with castrated rats receiving small doses of testosterone propionate, 5–50 μg./day, showed that the changes in the level of fructose and citric acid are much more prominent than the hormone-induced increases in size and weight of the corresponding rat organs. There are, of course, other sensitive androgenic tests such as, for instance, the capon comb test; however, results obtained with the capon test have no direct bearing on the problem of the quantitative relationship between the action of testosterone and the elaboration of seminal plasma in the male accessory organs, particularly in mammals. The fructose and citric acid tests are applicable to all species which normally secrete fructose and citric acid in semen, such as bull, ram, boar, stallion, guinea pig, rabbit and rat. On the other hand, they cannot be made use of in the capon since cock semen does not contain fructose or citric acid (Mann, 1948).

In a discussion of the significance of the testicular hormone as a factor which influences the composition of the seminal plasma, it is essential to bear in mind that in the living animal the activity of the male sex hormone is not solely dependent on the capacity of the testes to produce the hormone, but is in addition influenced indirectly by the action of the anterior pituitary hormones on the testes. In this paper hypophysectomy has been shown to be as effective as gonadectomy in abolishing the formation of fructose and citric acid in the accessory organs. It was possible to restore with gonadotrophin (from pregnant-mare serum) the fructose and citric acid formation in hypophysectomized rabbits.

Apart from the testes and the anterior pituitary, yet another organ, the pancreas, has now been shown to influence one seminal component, namely fructose. This influence is an indirect one: it is exerted by insulin on the level of fructose in semen. The link-up between the blood sugar level and seminal fructose has been studied in rabbits rendered diabetic by means of alloxan. Experimental diabetes caused a distinct rise in the level of fructose in semen. It was noticed, however, that the peak values were attained only after the hyperglycaemia has prevailed in the rabbit for some time. It is conceivable that some additional agents or mechanisms are involved in establishing the blood glucose: seminal fructose relation. Experiments on rabbits have shown that in severe diabetes the level of fructose in semen may exceed 4–5 times the control values, and that it can be brought back to normal with suitable doses of insulin. The rapid response to insulin, at any rate in the rabbit, was demonstrated by experiments in which collections of semen were taken shortly before and one day after, insulin treatment which led invariably to a sharp decline in the content of seminal fructose.

The behaviour of seminal fructose in diabetes must be taken as a strong indication that blood glucose acts as precursor of seminal fructose. The experiments on diabetic rabbits also lend new significance to the observations previously reported by Mann & Lutwak-Mann (1948) that small amounts of fructose are formed *in vitro* from glucose in the presence of minced seminal gland of bull. The conclusions drawn from experiments on rabbits probably apply also to other species, including man, where abnormally high values of seminal fructose were found to be associated with diabetes. However, the fact must not be overlooked that there are species such as, for example, sheep, where the normal blood sugar level is notoriously low, and which nevertheless exhibit normally a high level of fructose in semen.

SUMMARY

1. The formation and composition of seminal plasma is subject to control by several hormones.

2. The withdrawal of the male sex hormone through castration results in disappearance from the seminal plasma of two characteristic components, fructose and citric acid. The formation and secretion of both these substances can be successfully re-established by subcutaneous implantation or injection of pure testosterone.

3. A direct relationship exists between the amount of testosterone injected into castrated rats and the response of the accessory organs to produce fructose and citric acid. This makes it possible to use the quantitative assay of fructose and citric acid as a sensitive and simple 'hormone indicator test'.

4. Hypophysectomy produces effects similar to castration. The secretion of fructose and citric acid by the organs of the hypophysectomized animals can be induced by the administration of either gonadotrophin or testosterone.

5. In diabetes the content of fructose in semen is considerably increased. In alloxan-diabetic rabbits it was possible to demonstrate the existence of a relation between hyperglycaemia and the fructose level in semen. Insulin administration causes a fall in the level of fructose in the semen of diabetic rabbits.

6. The evidence is discussed which indicates that glucose acts as a precursor of seminal fructose in the animal body.

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A Note on the Copper-retaining Power of a Humic Acid from Peat Soil

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Bremner, Mann, Heintze & Lees (1946) have suggested that trace elements may be retained in soil as metallo-organic complexes with the soil organic matter. Lees (1948) estimated, from results obtained with whole cacao soils, that the copper retention is about 1400 μequiv. copper/g. soil organic matter. The copper-retaining power of 'humic acid' (the fraction of soil organic matter to which most of the trace element retention is presumably due) has now been measured directly.

EXPERIMENTAL

A peat soil from the San Juan Estate, Trinidad, was air-dried and a 100 g. sample repeatedly extracted with 500 ml. lots of 0·1 M-sodium oxalate at pH 7·0. Each lot of extractant was shaken with the soil intermittently over a period of 8 hr. after which the soil was allowed to settle for 16 hr.; the extract was then decanted and a fresh 500 ml. added to the residual soil. After five such extractions the combined extracts were acidified with conc. H_2SO_4 to a pH of approximately 2. The humic acid thus precipitated was centrifuged and then redissolved in 250 ml. water plus sufficient solid $NaHCO_3$ to bring the pH of the solution to 7·0. The humic acid solution was then filtered to remove clay and dialysed against repeatedly changed distilled water to remove salts. The humic acid was then reprecipitated by the addition of 4 vol. of 95% (v/v) ethanol and again filtered off. The precipitate was washed repeatedly with ethanol to remove as much water as possible and dried to a black cake in a desiccator over $CaCl_2$. This preparation of humic acid is based on the work of Bremner & Lees (1950).

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A 1% aqueous solution of dried, powdered, humic acid was treated in 1 ml. lots with 1 ml. 0·5 M-$Ca(NO_3)_2$ solution (to precipitate the humic acid and to saturate its base-exchange groups) plus 1·0, 1·5, 2·0, 2·5, 3·0 or 3·5 ml. $CuSO_4$ solution containing 10 μequiv. Cu/ml. solution,

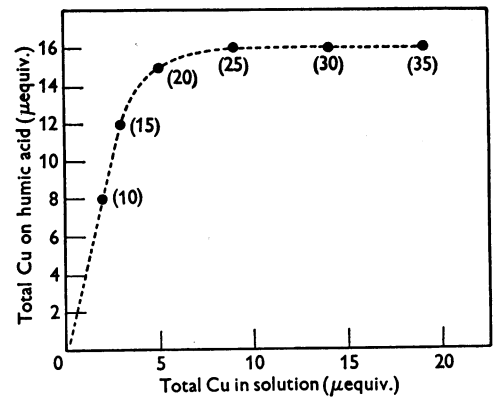

Fig. 1. The distribution of copper (added as $CuSO_4$) between 10 mg. humic acid and 11 ml. of $4·5 \times 10^{-2}$ M-$Ca(NO_3)_2$. (Each point is the mean of three determinations. Bracketed figures show total amount of copper (μequiv.) present.)

with sufficient distilled water to bring the final volume to 11 ml. After standing for 2 hr. the suspended humic acid was centrifuged and the Cu in the supernatant liquid was determined by the method of Sherman & McHargue (1942). The Cu retained by the humic acid was calculated by difference. The results obtained by the method are given in Fig. 1.